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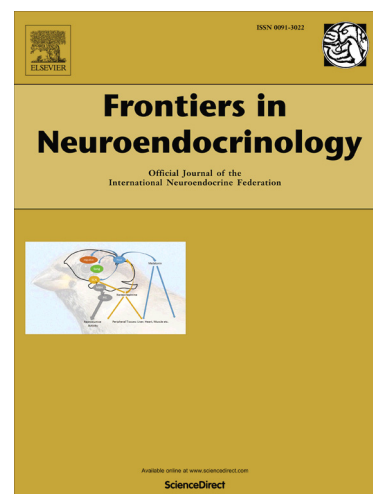
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Sleep & Metabolism: the multitasking ability of lateral hypothalamic inhibitory circuitries.

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Highlights

- Hypothalamus circuits support physiological regulation of homeostatic processes.
- Both peptidergic and GABA/Glutamate neurons from the lateral hypothalamus regulate metabolism and sleep-wake states.
- How single hypothalamic circuit controls sleep and metabolism remains unclear.
- The functional connectivity of sleep and metabolic circuits awaits further investigation.

Abstract

The anatomical and functional mapping of lateral hypothalamic circuits has been limited by the numerous cell types and complex, yet unclear, connectivity. Recent advances in functional dissection of input-output neurons in the lateral hypothalamus have identified subset of inhibitory cells as crucial modulators of both sleep-wake states and metabolism. Here, we summarize these recent studies and discuss the multi-tasking functions of hypothalamic circuitries in integrating sleep and metabolism in the mammalian brain.

Introduction

Sleep and wakefulness are two mutually exclusive behaviors. Sleep is “*a rapidly reversible state of (behavioral) immobility and greatly reduced sensory responsiveness to environmental stimuli*” (Siegel, 2008). Sleep-like states occur in lower vertebrates and throughout the animal kingdom, suggesting an ancient and strongly conserved mechanism necessary for primary and essential biological needs (but see (Siegel, 2008)). Sleep is important for brain maturation, cognitive processing and metabolite clearance in the brain. Sleep strongly depends on previous activity during wakefulness and prepares the brain and the body for future actions, yet our understanding of the neurobiological mechanisms underlying brain control of sleep-wake states is limited.

Here, we briefly summarize neuronal circuits underlying sleep-wake states with an emphasis on hypothalamic circuits. Other excellent reviews have recently summarized brain substrates of sleep-wake cycle (R. E. Brown et al., 2012; Fort et al., 2009) and food intake or metabolism (Sternson, 2013; Waterson and Horvath, 2015).

The control of sleep-wake state alternation is supported by distinct cellular networks (neuronal and non-neuronal cells) distributed across the central nervous system. The “stability” of this cycle is important for the proper functioning and the survival of the organism/species. In mammals, states of wakefulness, non-rapid eye movement (non-REM, or slow wave sleep) and rapid eye movement (REM, sometimes called paradoxical sleep) sleep are characterized by distinct electroencephalogram (EEG), electromyogram (EMG) and electrooculogram (EOG) signal features and cycle with both ultradian and circadian periods (Brown et al., 2012; Saper et al., 2010).

Wakefulness is characterized by high-frequency/low-amplitude cortical EEG oscillations (4-300 Hz), muscle activity and ocular movements.

After prolonged period of wakefulness, sleep pressure - which reflects a process called sleep homeostasis - increases and leads to the onset of NREM sleep. Cortical oscillations show both global and local oscillations composed of slow waves (< 1 Hz), high-amplitude delta oscillations (0.5-4 Hz), and spindles (9-15 Hz) accompanied by low muscle activity and the absence of ocular movement. REM sleep is a singular sleep state signalled by predominant EEG theta (6-9 Hz), and a complete disappearance of postural muscle tone (only muscle twitches persist), and fluctuation of the heart and breathing rates accompanied by rapid eye movements are frequently

observed during that state. While each of these states is mutually exclusive, sign of intertwined states occur under homeostatic challenge (e.g., sleep pressure or after learning) in local part of the cortex (Funk et al., 2016; Huber et al., 2004; Vyazovskiy et al., 2011).

Although the neurobiological mechanisms controlling the recurrence of these states across a 24 hour-period remain unclear, lesion, pharmacological and (opto)genetic studies strongly suggest that the onset, maintenance and termination of wake, NREM and REM sleep states relies on excitation/inhibition between distinct circuits distributed across the entire central nervous system (Fort et al., 2009; Luppi et al., 2016). In particular, unit recording in head-restrained animals revealed that wakefulness is associated with increased activity of the hypocretin/orexin (Hcr/ox)-expressing neurons in the lateral hypothalamus, the noradrenergic locus coeruleus (LC)-expressing neurons in the brainstem, the serotonergic dorsal raphe nuclei (DRN) in the brainstem, the histaminergic tuberomammillary nucleus (TMN) in the posterior hypothalamus, the cholinergic pedunculopontine (PPT) and laterodorsal tegmental (LDT) nuclei in the midbrain, as well as cholinergic neurons in the basal forebrain (reviewed in (R. E. Brown et al., 2012)). During NREM sleep, the activity of thalamo-cortico-thalamic circuits is highly synchronized and generates slow EEG oscillations as shown from *in vivo* recording of freely-moving animals. Similarly, inhibitory neurons in anterior hypothalamic (Alam et al., 1995; Zhang et al., 2015) and brainstem (Anaclet et al., 2014) structures are strongly active, however, their functional link to thalamo-cortical and cortical networks remains unclear. During REM sleep, inhibitory cells from the anterior (Lu et al., 2002) and the lateral (Clement et al., 2012; Jégo et al., 2013; Verret et al., 2003) hypothalamus, as well as glutamatergic and GABAergic neurons from the brainstem (Luppi et al., 2006) were found to be strongly active using immediate early gene detection and *in vivo* recordings.

Amassing literature highlighted a dual role for sleep-wake circuits in the brain. For instance, norepinephrine neurons from the locus coeruleus represent a major hub for wakefulness, but also control stress response and attention during cognitive processing (Aston-Jones and Bloom, 1981; Bouret and Sara, 2005). Similarly, sleep and sedation circuits located in the anterior hypothalamus (VLPO, LPOA, etc.)

concomitantly regulate body temperature (Schmidt, 2014; Szymusiak and Satinoff, 1981; Zhang et al., 2015). More caudally, neurons in the lateral hypothalamus (LH) expressing *hcrt/ox*, MCH, GABA and glutamate possess both sensing and controlling modalities, as their firing activity is strongly modulated by metabolic products both *in vitro* and *in vivo* (amino acids, glucose, etc.) (Burdakov et al., 2005; Karnani et al., 2011). Furthermore, the same cell types are also involved in the hypothalamic control of wakefulness, including *Hcrt* neurons and a subset of LH cells expressing VGAT, and both NREM and REM sleep, as exemplified by MCH cells (see below). These findings are discussed below and support the hypothesis that lateral hypothalamic circuits control both sleep and metabolism through multi-tasking networks. In a translational aspect, clinical and experimental studies report a high prevalence of metabolic syndrome associated with sleep disorders and vice versa. Patients with chronic sleep restriction, fragmented sleep or short sleep night present an increased risk for metabolic pathologies including diabetes and obesity, cardio-vascular risks, mood disorders and hormonal imbalances (J. A. Brown et al., 2015; Van Cauter et al., 2008).

This association suggests the existence of underlying circuitries that regulating both sleep/wake states and metabolism, as previously proposed (Adamantidis and de Lecea, 2008). *How sleep-wake circuit shares food intake or metabolic functions?* Both cellular/molecular and recent circuit evidence support these integrative properties amongst hypothalamic circuits. Here, we summarize recent findings linking sleep-wake state and metabolism and discuss lateral hypothalamic mechanisms underlying this patho-physiological associated symptom and point some future direction for the investigation for circuit multi-tasking properties.

Lateral hypothalamic (LH) circuitries in sleep-wake control

The LH is a homeostasis center that orchestrate food intake, metabolism balance, behaviours directed towards natural- (food, sex) and artificial- (drug) rewards (Bernardis and Bellinger, 1993; Saper et al., 2005; Stuber and Wise, 2016), and sleep-wake states (R. E. Brown et al., 2012; Saper et al., 2010). It contains multiple cell types with unique neurochemical profiles, vesicular transporter (s) (Collin et al., 2003; Rosin et al., 2003; Ziegler et al., 2002), connectivity (Ekstrand et al., 2014), membrane receptors (Leininger et al., 2009) or functions. In contrast to the laminar

structure of cortical or hippocampus networks, LH circuitries form an intricate local and extensive network of excitatory and inhibitory cells with no apparent anatomical features. Here, we will summarize recent studies on LH substrates of arousal, sleep and metabolism.

Electrophysiological recordings of LH cells across the sleep-wake cycle identified a wide variety of neurons activity of which correlates with NREM or REM sleep and/or wake states (R. E. Brown et al., 2012), suggesting the existence of neuronal (sub)populations with sleep- and wake-inducing properties. Indeed, neurons expressing Hypocretins/Orexins LH_{Hcr} - ($Hcr_{1,2}$, also known as Orexins_{A,B} (Sakurai et al., 1998)) and histamine (LH_{His}) represent wake-promoting systems (Lee et al., 2005): their cellular activity is typically low during quiet waking and highest during attention and active waking, and ceases firing almost completely during NREM and REM sleep. Accordingly, the activation of the Hcr system correlates with arousal/alertness associated with a stress response, stress-induced cocaine reinstatement, opioid addiction (Sakurai, 2014) and sensory stimulus (Hassani et al., 2016). Consistent with this correlative evidence, their optogenetic activation increases the probability of sleep-to-wake transitions (Adamantidis et al., 2007), defining an arousal circuit that is gated by sleep pressure (Carter et al., 2009) and relayed, at least in part, by LC_{NE} and TMN_{His} neurons (Eriksson et al., 2001; Schöne et al., 2014).

In contrast, REM sleep-active neurons have been described in the LH, including the GABA and MCH neurons using functional neuroanatomy (i.e., histological detection of neuronal activity marker protein c-Fos) and juxtacellular recording *in vivo* (Clement et al., 2012; Hassani et al., 2010; 2009) while NREM sleep-active neurons, that express galanin peptide, were recorded in the anterior hypothalamus of sleeping rats (Alam et al., 1995). Consistent with these correlative findings, recent studies demonstrated a sleep-promoting role for the MCH system. LH_{MCH} neurons express MCH peptide and several other peptides (nesfatin, CART, MGOP), together with the glutamate decarboxylase gene $GAD67/65$ that produces GABA (Clement et al., 2012; Elias et al., 2001). These findings suggested an inhibitory nature that has been recently confirmed by functional circuit mapping (Jego et al., 2013). Note that a study suggested that MCH neurons release glutamate in the septum (Chee et al., 2015), though the importance of this sub-population awaits further confirmation.

During a sleep rebound, a large number (60%) of c-Fos⁺ cells (marker of neuronal activity) are immuno-reactive for MCH peptide whereas Hcrt neurons are not. Indeed, MCH neurons show a discharges profile - maximal during REM sleep, low during NREM sleep and minimal during wakefulness - that is opposite to the activity of hcr/ox cells (Hassani et al., 2009). In addition, intracerebroventricular (icv) infusion of MCH in rats causes hypersomnia by dose-dependent increases of SWS (+70%) and REM sleep (+200%) amounts (Verret et al., 2003), later confirmed by chronic optogenetic stimulation (Konadhode et al., 2013; Tsunematsu et al., 2014), whereas a MCH-R1 antagonist has the opposite effect (Ahnaou et al., 2008). Consistent with these correlative findings, acute optogenetic activation of MCH neurons at the onset of REM sleep extended the duration of REM, but not non-REM sleep episodes (Jego et al., 2013). The latter result contrast with the previous studies and reveals the importance of acute vs chronic optogenetic stimulations. Jego et al used an acute stimulation that was time-locked to REM or NREM sleep episode, while others used chronic stimulation that were randomly delivered throughout the sleep-wake cycle, which ultimately induced a non-physiological sustained activity of MCH neurons, as compared to their REM sleep profile observed during juxtacellular recordings in head restrained rats (Hassani et al., 2009). In contrast, acute optogenetic silencing of MCH neurons reduced the frequency and amplitude of hippocampal theta rhythm, without affecting REM sleep duration, suggesting a backwards transition to NREM sleep, possibly through distributed inhibition of arousal center outside the hypothalamus.

Recent studies investigated the sleep and metabolic roles of other subsets of GABAergic cells in the LH using both functional and genetic tracing methods (Clement et al., 2012; Hassani et al., 2010; Herrera et al., 2015; Jennings et al., 2015). Note that the whole GABAergic neuron population in the LH consists of cell expressing VGAT, GAD 65/67, the long-form of the leptin receptor or MCH peptide. To date the precise co-expression of each of these markers within inhibitory cells of the LH remains unknown. Studies have used transgenic animals expressing the Cre recombinase in cells expressing the vesicular GABA transporter (VGAT), or detection of GAD 65/67 transcripts, hereafter refer as to LH_{VGAT} or LH_{GAD67}, respectively. Subsets of LH_{GABA} are active predominantly during wakefulness or REM sleep (state of cortical arousal and consciousness), as shown in juxta-cellular recording *in vivo* (Hassani et al., 2010). LH_{GAD67} neurons send descending projections

to the brainstem of the pons, where neurons involved in promoting wake or regulating REM sleep states are located (Clement et al., 2012), as well as ascending projections to the cerebral cortex (Manns et al., 2000). Collectively, these results suggested a strong anatomical heterogeneity amongst LH_{GABA} neurons in controlling brain states. Although juxta-cellular recording suggested the existence of REM sleep active LH_{VGAT} neurons (Hassani et al., 2010), no experimental evidence has identified the subpopulation responsible for this effect yet. Therefore, we will focus our attention on their control of arousal.

Consistent with their activity during wakefulness, acute optogenetic activation of LH_{VGAT} neurons promotes rapid arousal (Herrera et al., 2015). More specifically, those that mono-synaptically project to the thalamus reticular nuclei, hereafter refer to as LH_{VGAT}-TRN_{GABA} circuit, represent a major arousal circuit of the hypothalamus (Herrera et al., 2015) (Figure 2). In contrast, its optogenetic activation during REM sleep had no effect, suggesting that the LH_{VGAT}-TRN_{GABA} circuit is involved in NREM sleep, but not REM sleep-to-wake transitions. In contrast, a subset of LH_{VGAT} cell projecting to the locus coeruleus induced arousal response independent of the brain states of the animal (Herrera et al., 2015). Through its direct feed-forward disinhibition of thalamo-cortical network, this LH_{VGAT}-TRN_{GABA} arousal circuit showed significant potency at inducing emergence from deep anaesthesia (Herrera et al., 2015). Interestingly, this effect was faster than pharmacogenetic activation of LC_{NE} neurons in inducing arousal and emergence from anaesthesia (Vazey and Aston-Jones, 2014), suggesting that the LH_{VGAT}-TRN_{GABA} circuit represents a potent arousal circuit that promotes arousal from NREM, but not REM, sleep. Overall, this latter result support the idea that there is a high specification, rather than redundancy, amongst arousal circuits in the brain.

LH circuitries: multitasking sleep/arousal and metabolism ?

Interestingly, while acute optogenetic stimulation of LH_{VGAT} cells during RREM sleep provoked rapid arousal (Herrera et al., 2015), their chronic optical activation during wakefulness induced a sustained food intake response (Jennings et al., 2015). These findings provide a causal evidence for a dual role of these cells in sleep and metabolism.

Appetite is regulated by the interaction between metabolic and hormonal signals and

the central nervous system. The hypothalamus regulates energy homeostasis (i.e. food intake and energy expenditure) by sensing circulating hormones including leptin, ghrelin, glucose, amino acids, and integrating autonomic, endocrine and environmental signals into coherent goal-directed behaviors (Sternson, 2013; Waterson and Horvath, 2015). Leptin inhibition of arcuate neurons that coexpress neuropeptide Y (NPY) and agouti-related peptide (AGRP) and excitation of proopiomelanocortin (POMC) neurons, that also co-express cocaine- and amphetamine-related transcripts (CART), originally define the "first-order" circuits of food intake (Schwartz et al., 2000; Sternson, 2013) (but see Ref. (Chen et al., 2015; Waterson and Horvath, 2015)). Additional circulating factors reveal a more complex mechanism, as shown by the discovery of ghrelin, an appetite-stimulating hormone from the gut that has the opposite effect. Thus, activation of POMC/CART and NPY/AGRP neurons has anorexigenic and orexigenic properties, respectively. Thus, "First order" arcuate neurons responsible for energy-sensing project to "Second order" lateral hypothalamus (LH), where environmental, compulsive, rewarding/hedonic signals - from extra-hypothalamic afferences including PFC, amygdala, BF, BNST, PB, LS, VP, etc. - are integrated to the homeostatic component of the initial response into a coordinated innate/purposive/directed behavior (Seeley and Woods, 2003). "Second order" neurons include cells in the medial and lateral hypothalamic areas producing Hcrt, MCH, CART, Neurotensin, Nesfatin, endocannabinoids, and in a broader view LH_{GABA} and LH_{Glu} neurons. Note that "Second order" neurons in the LH also have sensing capabilities (Brown et al., 2015; Burdakov et al., 2005), and may shunt arcuate nucleus mediated metabolic control. Whether and how "Second order" circuit have a preponderant role on food intake than the "First order" circuit remains unclear and await further investigation. Here, we will focus on the LH_{GABA} cells since others systems have not been evidenced or are reviewed elsewhere (Brown et al., 2015; Koch et al., 2015; Sternson, 2013).

Electrical and pharmacological activation of LH neurons reveal their participation to food intake (DELGADO and ANAND, 1953) and reinforcement process (OLDS and MILNER, 1954), suggesting multiple modulation of reward processing from initial consumption to behavioral/instrumental conditioning and stereotypic behaviors depending on the topography, techniques and intensity of the perturbation, the species considered and inter-individual response to goals (Flagel et al., 2011).

A collection of recent studies unravel the complex nature of LH_{VGAT} cells based on their expression of VGAT, GAD, MCH or leptin long-form receptor (LepRb) markers (Goforth et al., 2014; Jegu et al., 2013; Jennings et al., 2015; Nieh et al., 2015). In summary, LH_{VGAT} cells are not MCH⁺, while most of MCH cells are GAD⁺ (Clement et al., 2012; Jegu et al., 2013; Sapin et al., 2009). How many VGAT⁺ cells are also positive for GAD is unknown at the moment. There is an increasing appreciation that VGAT may not be essential for loading GABA into synaptic vesicles, and other vesicular transporters such as VMAT2 could also mediate this function (Tritsch et al., 2012). Therefore, it is likely that studies that target LH inhibitory neurons using VGAT (e.g., using VGAT-cre mice) do not fully capture the roles of all inhibitory neurons in the area.

Although LH_{MCH} are not VGAT⁺, we will summarize their participation of metabolism and appetite. Amongst the numerous roles for MCH neurons, their involvement in feeding behavior and energy homeostasis are by far the best documented (reviewed in (Pissios and Maratos-Flier, 2003; Presse et al., 2014)). MCH has acute short-term orexigenic properties; in fact, the MCH system is upregulated after fasting. Mice overexpressing MCH develop mild obesity and hyperphagia, whereas genetic inactivation of MCH neurotransmission (MCH KO, MCH-R1 KO) and ablation of MCH neurons in mice provokes leanness, hyperactivity and increased metabolic rate (Pissios, 2009). Interestingly, genetic deletion of the MCH system in leptin-deficient (*ob/ob*) obese mice does not alter hyperphagia of *ob/ob* animals, but induces a dramatic reduction in body fat secondary to a marked increase in energy expenditure (Segal-Lieberman et al., 2003).

MCH modulation of the flow of cerebrospinal fluid was recently proposed as a possible mechanism for MCH neurons control of metabolism. In two separate studies, Conductier and colleagues found that MCH peptide positively controlled cilia beat frequency from ependymal cell of the ventral third ventricle. It is suggested that MCH neurons firing increases the flow of cerebrospinal fluid through the cerebral ventricles. In contrast, the lack of a functional MCH receptor increases ventricular size, presumably due to altered cerebral fluid flow through the ventricles. These findings were confirmed by optogenetic activation and silencing of MCH neurons *in vitro* which increase and decrease CSF flow, respectively (Conductier et al., 2013).

Ultimately, these results suggest that MCH-mediated modulation of ciliary beating and CSF flow participates to the response to metabolic, neuro-hormonal, and neuro-immune changes that would result in metabolic dysfunction (e.g. obesity, etc.) in the absence of a functional MCH system.

Beside this metabolic effect, optogenetic activation of MCH neurons has recently been shown to support the post-ingestive rewarding effect following food intake, through an increased striatal dopamine levels, while MCH neurons ablation blunts this effect (Domingos et al., 2013). This is consistent with a role for MCH peptide in initiating and maintaining behaviors that are under the control of conditioned reinforcers (Sherwood et al., 2012) and MCH participation in cue-evoked overeating of sucrose under satiety (Sherwood et al., 2015).

These studies collectively demonstrate that activation of the MCH system dampens energy expenditure and adds up to the central and peripheral pathways (Brown et al., 2015) involving MCH neurons in metabolism and reward value. Yet, the link between NREM/REM sleep, metabolism and goal-directed behaviours remains unclear. To date, there is no report of a feeding or metabolic response upon optogenetic manipulation of MCH neurons, or their cellular activity (e.g., discharge rate or calcium transient). A recent study observed that MCH cell activity correlates with novelty exploration, while they are inhibited by stress (Gonzales et al 2016), however, their precise cellular activity during food intake or negative energy balance - e.g., fasting, which has been shown to increase MCH neurons (Qu et al, Lembo) - remains to be characterized using imaging technologies.

Beside the LH_{MCH} cells that are predominantly GAD⁺, LH_{VGAT} cells (these are MCH⁺) have also been identified as a crucial component of appetite. Local manipulation of LH neurons by infusion of GABA_A agonist mediates suppression of food intake and decrease body weight, while GABA_A antagonists actually elicit eating in sated rats (Bernardis and Bellinger, 1993). These results contrast with recent optogenetic studies. Indeed, optogenetic stimulation showed that activation of LH_{VGAT} induced food intake, consistent with electrical studies, and optical self-stimulation (Jennings et al., 2015), while activation of LH_{vglut2} has the opposite effect (Jennings et al., 2013; Stamatakis et al., 2016). Genetic ablation of these cells produce behavior consistent

with gain-of-function approaches (Jennings et al., 2015; Stamatakis et al., 2016). Together these findings suggest that, first, modulation of neurotransmitters such as GABA and Glutamate can generate fast feeding responses such as those observed with manipulation of "*First order*" neurons of the arcuate nuclei; second, genetically distinct GABAergic and glutamatergic LH subpopulations can produce a bidirectional output signals (i.e., opposing behavioral phenotypes); and third, this may reflect the moment-to-moment balance in the activity of glutamate and GABA within the LH.

A circuit perspective on multi-tasking circuits

As summarized above, many circuits of the brain shared one, two or more functions, as do LH neurons control both arousal and food intake, as possibly other unknown functions, in the mammalian brain (Table 1). Although there is likely an anatomical and functional disparity between LH_{GAD} and LH_{VGAT} neurons, in the following section, we will refer to LH_{GABA} cells in general for clarity of our speculation, despite obvious diversity amongst those cells.

Function modulated by brain states

Consistent with a multi-tasking role of LH cells in sleep and metabolism, challenging one or the other often exacerbate metabolism or sleep homeostat, respectively. For instance, LH_{hcr/ox} neurons are important to maintain arousal require for food seeking-behaviour (Yamanaka et al., 2003) and exacerbate depression-like behavior in fasted animals (Lutter et al., 2008). Similar findings are also true for LH_{MCH} modulation of REM sleep during negative energy balance status (Willie et al., 2008). In line with these findings, recent studies summarized here already support a possible multi-tasking role for LH_{GABA} neurons in arousal and metabolism. Indeed, acute (< 10 s) optogenetic activation of LH_{VGAT} neurons during NREM sleep is sufficient to induce rapid arousal (Herrera et al., 2015) with latency similar to LC_{NE} arousal neurons (Carter et al., 2010), while their chronic activation promote sustained wakefulness and eventually increases food intake in sated animals (see below; Stuber and Wise, 2016).

Although this is purely speculative at the moment, the anatomo-functional diagram of LH_{GABA} cell inputs-outputs could contribute to such multi-tasking capabilities. Distinct LH_{GABA} cell targets could potentially mediate either arousal and/or food

intake. Optogenetic activation of LH_{VGAT}-TRN_{GABA} circuit did recapitulate the major arousal effect of LH_{VGAT} somatic activation, however, it is unlikely that the TRN target mediates food intake. Instead, other LH_{GABA} targets, in particular the lateral habenula (LHb), ventral-tegmental area, local hypothalamic circuits, periventricular thalamic nuclei, or brainstem nuclei represent functional targets that may mediate the feeding and rewarding effects associated with LH_{VGAT} cells activation (González et al., 2016; Kempadoo et al., 2013; Nieh et al., 2015; Stuber and Wise, 2016; Labouebe et al., 2016). Note that this was recently shown for LH_{vglut2}-LHb circuits (Stamatakis et al., 2016). Of interest, the periventricular thalamic nuclei that promote both arousal (although at a slower speed than TRN; Herrera et al., 2015) and food intake (Haight et al., 2015; Stratford and Wirtshafter, 2013) may represent a convergent LH_{GABA} target for sleep and metabolism multi-tasking. Similarly, brainstem targets, including locus coeruleus (Carter et al., 2010) and parabrachial nucleus (Carter et al., 2013), may also modulate both arousal and appetite response, possibly through local circuits. Whether multi-tasking LH_{GABA} cells belong to distinct cell clusters or a single cluster with multiple projections remains to be investigated.

Alternatively and in light of the functional anatomy of hypothalamic neurons and LH_{GABA} cells in particular, two circuits-based mechanisms underlying sleep and metabolic control could be speculated. First, the activity of LH_{GABA} cell cluster governing sleep and metabolism is *sequentially* organized. In this scheme, they first elicit arousal from sleep, which is permissive to the elaboration of food intake behavior, while their prolonged activity ultimately induced food intake. Alternatively, their activation during wakefulness consolidates arousal, as well as attention and/or integration of environmental factors relevant to consumption behaviors (e.g., the value of the reward-associated cue; see above). This would ultimately lead to an appropriate goal-directed behaviour (food intake) and further adjustment of the metabolic state of the organism. The fact that activation of LH_{GABA} cells elicits appetite in sated animals suggests that this food intake response is not a consequence of the animal being awake, but rather a specific goal response. Upon satiety, the activity of LH_{GABA} cells is progressively turned OFF (or undergo specific re-organization of discharge patterns), sequentially or not, to allow a (post-prandial) rest period and the onset of sleep (González et al., 2016). Second, the activity of LH_{GABA} cell cluster governing sleep and metabolism is organized in *parallel*. In this case, and

according to the modulatory circuit model of the brain, LH_{GABA} cell clusters targets are active simultaneously to promote both arousal and food intake during wakefulness. Note that several factors are involved, including: 1) unknown functions modulated by these clusters - visual attention, motor coordination, etc. - which may reinforce the selectivity of the behavioral response; and 2) sequential and parallel pathways are not mutually exclusive and may occur simultaneously as well to potentiate a given behavioral response.

Both sequential and parallel mechanisms have been described in the central nervous system, including the "First order" and "Second order" neurons in food intake (Schwartz et al., 2000), or the ascending reticular activating system in arousal (Brown et al., 2012). Despite the sequential or parallel activation of LH_{GABA} cell cluster, the synaptic mechanism remains unclear. Direct postsynaptic GABA_{A/B}-mediated inhibition can result in an indirect net excitation, through dis-inhibition, of arousal and/or appetite circuits or a direct inhibition of sleep centers and appetite-suppressing systems (Carter et al., 2013). Accordingly, a systematic assessment of vesicular content/release and neurotransmission amongst the circuit of interest, one synapse at a time, reveal important rules of communication, as shown for LH_{MCH} and LH_{hcr/ox} neurons (Jego et al., 2013; Schöne et al., 2014).

Inputs-outputs LH_{GABA} map

Part of what defines a sequential vs parallel mechanism is the anatomical and functional inputs-outputs diagram of the LH_{GABA} cluster considered. Although, the inputs mapping to LH_{GABA} cells remains sparsely described, we hypothesize that LH_{VGAT} neurons represent a small cluster of cells that are the monosynaptic targets of many cortical and subcortical circuits, similarly to LH_{MCH} or LH_{hcr} (see González et al., 2016; Sakurai et al., 2005; van den Pol et al., 2004). According to the functional anatomy of neuromodulatory systems of the brain, they send widespread projections to cortical and subcortical structures (Clement et al., 2012; Herrera et al., 2015; Jego et al., 2013; Jennings et al., 2013; Nieh et al., 2015; Stuber and Wise, 2016). Thus, defining topographical or functional clusters of LH_{GABA} cells - should these belong to a LH_{VGAT} and LH_{GAD} clusters or not - controlling arousal and metabolic is an important experimental task. Unravelling their specific input-output connectivity and the temporal sequence of their cellular activity across spontaneous sleep-wake and metabolic states (including food intake) is now a possible with the use of recent

imaging technologies. Indeed, subsets of VGAT cells has revealed additional functional clusters that not only signal food intake, but also distance to and spatial localization of reward targets (Jennings et al., 2015). Imaging effort will undoubtedly define the rules of multi-tasking amongst neural circuits. In these functional circuits dissection, one must bear in mind that random, or sustained optogenetic activation of circuit that are normally not active during innate or learned behaviors may not reveal the true underlying neurobiological mechanism, but rather induced artificial, sometimes almost identical, neurophysiological response. In other words, optogenetic stimulation parameter should be designed based on the precise firing of the targeted cells during food intake or across sleep-wake states.

Despite a strong modulation of sleep and metabolism, the lateral hypothalamus also supports other survival functions including fight-or-flight response, stress, anxiety, maternal behavior reproduction and reward. Therefore, one can further speculate that a single LH circuit integrates additional physiological functions beside sleep and metabolism, however the underlying mechanisms await further investigation.

Perspectives

The LH_{GABA} cells provide a working model to study multi-tasking amongst neural circuit in the mammalian brain. Although our understanding of LH_{GABA} cell control of sleep and metabolism has progressed over the last years, several challenges remain. One of them is to define whether shared functions are integrated within the same cellular network, or whether it relies on separate subsets of strongly connected neurons within the LH. Equally important if to assess the importance of inputs-outputs connectivity in delineating distinct or shared cluster cells controlling both sleep and metabolism. Whether these are organized in a "convergent-divergent" input-output or (linear) reciprocal model remains to be determined. The synergistic combination of (opto)genetic - both optical imaging and manipulating capabilities - with circuit-mapping and precise behavioral screening will decipher the logic rules of LH circuit functioning and, ultimately, identify pathophysiological mechanisms for combined therapeutic strategies.

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Table 1: Summary of behavioural responses to activation of distinct LH cell types.

Cell types	Energy expenditure (metabolic rate)	Food intake	Arousal	NREM sleep	REM sleep
MCH	↘	↗	↔	↗	↗
Hcr/Ox	↗	↗	↗	↘	↘
VGAT	-	↗	↗	↘	↔
VGlut ₂	-	↘	-	-	-

Figure legend**Figure 1. Optogenetic probing of the role of LH_{Hcrt/Ox} and LH_{Hcrt/Ox} neurons in arousal and REM sleep *in vivo*.**

a, Fluorescent images showing the ChR2-mCherry to LH_{Hcrt/Ox} neurons using a lentivirus strategy. b, Fluorescent images showing cell-specific cre expression (red) in MCH neurons (green) in the LH of [MCH::Cre X tdtomato reporter] mouse. Plain and open arrowhead indicates Cre⁺ and Cre⁻ MCH neurons, respectively. c, 5 ms light pulse trains (20 Hz; 473 nm) evoked reliable firing of action potential with high temporal precision in ChR2-expressing Hcrt neurons *in vitro*. d, ChETA-eYFP-expressing MCH neurons respond to optical stimulation in anesthetized transduced mice. e, Optogenetically-induced and spontaneous action potentials of MCH neurons show similar waveform profiles. f, Optogenetic activation of ChR2-expressing LH_{Hcrt/Ox} neurons promotes sleep-to-wake transitions in ChR2 animals (N=7) compared to controls (N=6). ***, $p < 0.0001$. g, Optogenetic activation (20 Hz) of ChR2-expressing LH_{MCH} neurons promotes REM sleep in ChR2 animals (N=6) compared to controls (N=6). *, $p < 0.05$, **, $p < 0.01$. h, Optogenetic silencing of MCH neurons during REM sleep induced slow theta oscillation in eNpHR3.0 animals, suggesting a REM -to-NREM sleep "backward" transition. i, Quantification of slow theta oscillations shown in (h) **, $p < 0.01$. *Modified from*

Figure 2: Hypothalamic feed-forward inhibition of thalamocortical network controls arousal and consciousness.

a, Schematic of genetic targeting and surgical implantation of opto-tetrode (32 ch) in the TRN. EEG/EMG are not shown b, Photomicrographs of coronal section showing ChETA-EYFP-expressing LHGABA terminals (top) intermingled with GAD-67+ cells in the posterior TRN (bottom). Scale bar: 20 μ m. c, Average BIC-sensitive IPSCs amplitude \pm S.E.M of TRN cells upon optical stimulation of ChETA-expressing LHGABA terminals; Inset: Whole cell voltage-clamp recordings show robust BIC-sensitive (back traces) inward photocurrents (grey traces) evoked by single 5-ms 473 nm light pulses. d, Representative EEG/EMG/TRN Unit recordings during spontaneous NREM sleep-to-wake transition in freely-moving mouse. Note the transient quiescence of TRN cell preceding behavioural transition (green box). e, Mean latencies of NREM sleep-to-wake transitions upon optogenetic stimulation in ChETA (N=7) and control animals (N=5). **, $P < 0.001$, ***, $P < 0.0001$. f,

Representative EEG/EMG recordings and EEG power spectrum heat map before, during and after single 5-s optical stimulation (blue bars) in ChETA-YFP (right) animals during deep anaesthesia (1.5% isoflurane). Note the muscle tone that occur in ChETA-YFP (2 out of 5 animals). Modified from

Figure 3: Hypothalamic feed-forward inhibition of thalamocortical network controls arousal and consciousness.

Schematic of the feed-forward inhibitory LH_{GABA}-TRN-Thalamocortical arousal circuit. Inhibitory inputs (red) from the hypothalamus directly control the activity of TRN cell, which further exert a strong inhibitory control over excitatory (green) thalamocortical (TC) and cortico-thalamic (CT) cells. *INSET* shows the anatomical inputs to inhibitory cells of the LH and possible parallel and sequential multi-tasking outputs. Note that the chemical nature of inhibitory subpopulations of the LH remains only partially known.

References

- Adamantidis, A., de Lecea, L., 2008. Sleep and metabolism: shared circuits, new connections. *Trends Endocrinol. Metab.* 19, 362–370.
- Adamantidis, A.R., Zhang, F., Aravanis, A.M., Deisseroth, K., de Lecea, L., 2007. Neural substrates of awakening probed with optogenetic control of hypocretin neurons. *Nature* 450, 420–424.
- Ahnaou, A., Drinkenburg, W., Bouwknecht, J.A., 2008. Blocking melanin-concentrating hormone MCH 1 receptor affects rat sleep–wake architecture. *Eur. J. Neurosci.* 579, 177–188.
- Alam, M.N., McGinty, D., Szymusiak, R., 1995. Neuronal discharge of preoptic/anterior hypothalamic thermosensitive neurons: relation to NREM sleep. *Am. J. Physiol.* 269, R1240–9.
- Anaclet, C., Ferrari, L., Arrigoni, E., Bass, C.E., Saper, C.B., Lu, J., Fuller, P.M., 2014. The GABAergic parafacial zone is a medullary slow wave sleep-promoting center. *Nat. Neurosci.* 17, 1217–1224.
- Aston-Jones, G., Bloom, F.E., 1981. Activity of norepinephrine-containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *J. Neurosci.* 1, 876–886.
- Bernardis, L.L., Bellinger, L.L., 1993. The lateral hypothalamic area revisited: neuroanatomy, body weight regulation, neuroendocrinology and metabolism. *Neurosci Biobehav Rev* 17, 141–193.
- Bouret, S., Sara, S.J., 2005. Network reset: a simplified overarching theory of locus coeruleus noradrenaline function. *Trends in Neurosciences* 28, 574–582.
- Brown, J.A., Woodworth, H.L., Leininger, G.M., 2015. To ingest or rest? Specialized roles of lateral hypothalamic area neurons in coordinating energy balance. *Front Syst Neurosci* 9, 9.
- Brown, R.E., Basheer, R., McKenna, J.T., Strecker, R.E., McCarley, R.W., 2012. Control of sleep and wakefulness. *Physiological Reviews* 92, 1087–1187.
- Burdakov, D., Gerasimenko, O., Verkhratsky, A., 2005. Physiological changes in glucose differentially modulate the excitability of hypothalamic melanin-concentrating hormone and orexin neurons in situ. *J. Neurosci.* 25, 2429–2433.
- Carter, M.E., Adamantidis, A., Ohtsu, H., Deisseroth, K., de Lecea, L., 2009. Sleep homeostasis modulates hypocretin-mediated sleep-to-wake transitions. *J. Neurosci.* 29, 10939–10949.
- Carter, M.E., Soden, M.E., Zweifel, L.S., Palmiter, R.D., 2013. Genetic identification of a neural circuit that suppresses appetite. *Nature* 503, 111–114.
- Carter, M.E., Yizhar, O., Chikahisa, S., Nguyen, H., Adamantidis, A., Nishino, S., Deisseroth, K., de Lecea, L., 2010. Tuning arousal with optogenetic modulation of locus coeruleus neurons. *Nat. Neurosci.* 13, 1526–1533.
- Chee, M.J.S., Arrigoni, E., Maratos-Flier, E., 2015. Melanin-concentrating hormone neurons release glutamate for feedforward inhibition of the lateral septum. *J. Neurosci.* 35, 3644–3651.
- Chen, Y., Lin, Y.-C., Kuo, T.-W., Knight, Z.A., 2015. Sensory detection of food rapidly modulates arcuate feeding circuits. *Cell* 160, 829–841.
- Clement, O., Sapin, E., Libourel, P.A., Arthaud, S., Brischoux, F., Fort, P., Luppi, P.H., 2012. The Lateral Hypothalamic Area Controls Paradoxical (REM) Sleep by Means of Descending Projections to Brainstem GABAergic Neurons. *J. Neurosci.* 32, 16763–16774.
- Collin, M., Bäckberg, M., Ovesjö, M.-L., Fisone, G., Edwards, R.H., Fujiyama, F., Meister, B., 2003. Plasma membrane and vesicular glutamate transporter

- mRNAs/proteins in hypothalamic neurons that regulate body weight. *Eur. J. Neurosci.* 18, 1265–1278.
- Conductier, G., Martin, A.O., Risold, P.Y., Jegu, S., Lavoie, R., Lafont, C., Mollard, P., Adamantidis, A., Nahon, J.-L., 2013. Control of ventricular ciliary beating by the melanin concentrating hormone-expressing neurons of the lateral hypothalamus: a functional imaging survey. *Front Endocrinol (Lausanne)* 4, 182.
- DELGADO, J.M.R., ANAND, B.K., 1953. Increase of food intake induced by electrical stimulation of the lateral hypothalamus. *Am. J. Physiol.* 172, 162–168.
- Domingos, A.I., Sordillo, A., Dietrich, M.O., Liu, Z.-W., Tellez, L.A., Vaynshteyn, J., Ferreira, J.G., Ekstrand, M.I., Horvath, T.L., de Araujo, I.E., Friedman, J.M., 2013. Hypothalamic melanin concentrating hormone neurons communicate the nutrient value of sugar. *Elife* 2, e01462.
- Ekstrand, M.I., Nectow, A.R., Knight, Z.A., Latcha, K.N., Pomeranz, L.E., Friedman, J.M., 2014. Molecular profiling of neurons based on connectivity. *Cell* 157, 1230–1242.
- Elias, C.F., Lee, C.E., Kelly, J.F., Ahima, R.S., Kuhar, M., Saper, C.B., Elmquist, J.K., 2001. Characterization of CART neurons in the rat and human hypothalamus. *J. Comp. Neurol.* 432, 1–19.
- Eriksson, K.S., Sergeeva, O., Brown, R.E., Haas, H.L., 2001. Orexin/hypocretin excites the histaminergic neurons of the tuberomammillary nucleus. *J. Neurosci.* 21, 9273–9279.
- Flagel, S.B., Clark, J.J., Robinson, T.E., Mayo, L., Czuj, A., Willuhn, I., Akers, C.A., Clinton, S.M., Phillips, P.E.M., Akil, H., 2011. A selective role for dopamine in stimulus-reward learning. *Nature* 469, 53–57.
- Fort, P., Bassetti, C.L., Luppi, P.H., 2009. Alternating vigilance states: new insights regarding neuronal networks and mechanisms. *Eur. J. Neurosci.* 29, 1741–1753.
- Funk, C.M., Honjoh, S., Rodriguez, A.V., Cirelli, C., Tononi, G., 2016. Local Slow Waves in Superficial Layers of Primary Cortical Areas during REM Sleep. *Curr. Biol.* 26, 396–403.
- Goforth, P.B., Leininger, G.M., Patterson, C.M., Satin, L.S., Myers, M.G., 2014. Leptin acts via lateral hypothalamic area neurotensin neurons to inhibit orexin neurons by multiple GABA-independent mechanisms. *J. Neurosci.* 34, 11405–11415.
- González, J.A., Iordanidou, P., Strom, M., Adamantidis, A., Burdakov, D., 2016. Awake dynamics and brain-wide direct inputs of hypothalamic MCH and orexin networks. *Nat Commun* 7, 11395.
- Haight, J.L., Fraser, K.M., Akil, H., Flagel, S.B., 2015. Lesions of the paraventricular nucleus of the thalamus differentially affect sign- and goal-tracking conditioned responses. *Eur. J. Neurosci.* 42, 2478–2488.
- Hassani, O.K., Henny, P., Lee, M.G., Jones, B.E., 2010. GABAergic neurons intermingled with orexin and MCH neurons in the lateral hypothalamus discharge maximally during sleep. *Eur. J. Neurosci.* 32, 448–457.
- Hassani, O.K., Krause, M.R., Mainville, L., Cordova, C.A., Jones, B.E., 2016. Orexin Neurons Respond Differentially to Auditory Cues Associated with Appetitive versus Aversive Outcomes. *J. Neurosci.* 36, 1747–1757.
- Hassani, O.K., Lee, M.G., Jones, B.E., 2009. Melanin-concentrating hormone neurons discharge in a reciprocal manner to orexin neurons across the sleep-wake cycle 106, 2418–2422.
- Herrera, C.G., Cadavieco, M.C., Jegu, S., Ponomarenko, A., Korotkova, T., Adamantidis, A., 2015. Hypothalamic feedforward inhibition of thalamocortical

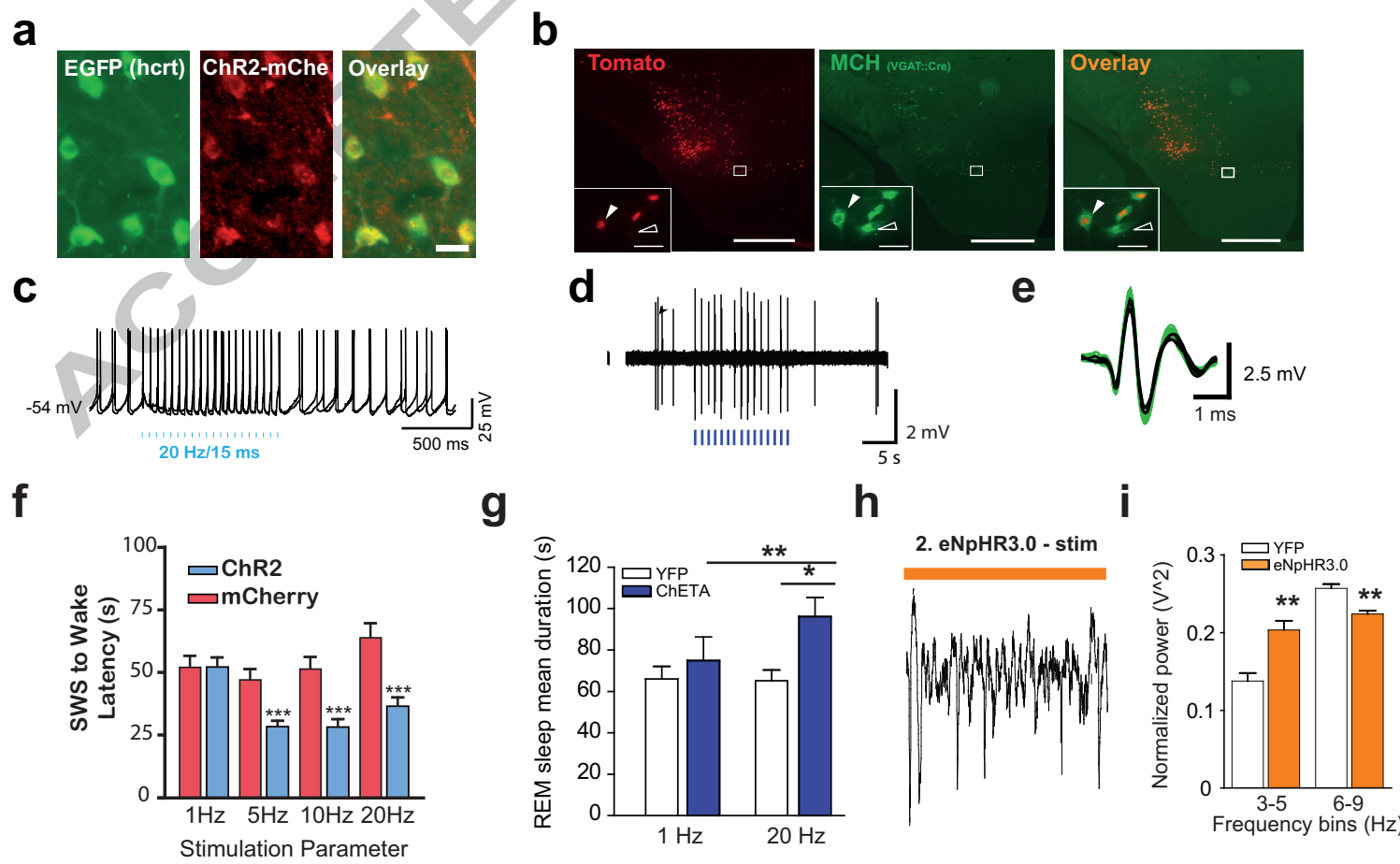
- network controls arousal and consciousness. *Nat. Neurosci.* 19, 290-298.
- Huber, R., Ghilardi, M.F., Massimini, M., Tononi, G., 2004. Local sleep and learning. *Nature* 430, 78–81.
- Jego, S., Glasgow, S.D., Herrera, C.G., Ekstrand, M., Reed, S.J., Boyce, R., Friedman, J., Burdakov, D., Adamantidis, A.R., 2013. Optogenetic identification of a rapid eye movement sleep modulatory circuit in the hypothalamus. *Nat. Neurosci.* 16, 1637–1643.
- Jennings, J.H., Rizzi, G., Stamatakis, A.M., Ung, R.L., Stuber, G.D., 2013. The inhibitory circuit architecture of the lateral hypothalamus orchestrates feeding. *Science* 341, 1517–1521.
- Jennings, J.H., Ung, R.L., Resendez, S.L., Stamatakis, A.M., Taylor, J.G., Huang, J., Veleta, K., Kantak, P.A., Aita, M., Shilling-Scriver, K., Ramakrishnan, C., Deisseroth, K., Otte, S., Stuber, G.D., 2015. Visualizing hypothalamic network dynamics for appetitive and consummatory behaviors. *Cell* 160, 516–527.
- Karnani, M.M., Apergis-Schoute, J., Adamantidis, A., Jensen, L.T., de Lecea, L., Fugger, L., Burdakov, D., 2011. Activation of central orexin/hypocretin neurons by dietary amino acids. *Neuron* 72, 616–629.
- Kempadoo, K.A., Touriño, C., Cho, S.L., Magnani, F., Leininger, G.-M., Stuber, G.D., Zhang, F., Myers, M.G., Deisseroth, K., de Lecea, L., Bonci, A., 2013. Hypothalamic neurotensin projections promote reward by enhancing glutamate transmission in the VTA. *J. Neurosci.* 33, 7618–7626.
- Koch, M., Varela, L., Kim, J.G., Kim, J.D., Hernández-Nuño, F., Simonds, S.E., Castorena, C.M., Vianna, C.R., Elmquist, J.K., Morozov, Y.M., Rakic, P., Bechmann, I., Cowley, M.A., Szigeti-Buck, K., Dietrich, M.O., Gao, X.-B., Diano, S., Horvath, T.L., 2015. Hypothalamic POMC neurons promote cannabinoid-induced feeding. *Nature* 519, 45–50.
- Konadhode, R.R., Pelluru, D., Blanco-Centurion, C., Zayachivsky, A., Liu, M., Uhde, T., Glen, W.B., van den Pol, A.N., Mulholland, P.J., Shiromani, P.J., 2013. Optogenetic stimulation of MCH neurons increases sleep. *Journal of Neuroscience* 33, 10257–10263.
- Labouebe G, Boutrel B, Tarussio D, Thorens B. 2016. Glucose-responsive neurons of the paraventricular thalamus control sucrose-seeking behavior. *Nat Neurosci.* 19(8):999-1002.
- Lee, M.G., Hassani, O.K., Jones, B.E., 2005. Discharge of identified orexin/hypocretin neurons across the sleep-waking cycle. *J. Neurosci.* 25, 6716–6720.
- Leininger, G.M., Jo, Y.-H., Leshan, R.L., Louis, G.W., Yang, H., Barrera, J.G., Wilson, H., Opland, D.M., Faouzi, M.A., Gong, Y., Jones, J.C., Rhodes, C.J., Chua, S., Diano, S., Horvath, T.L., Seeley, R.J., Becker, J.B., Münzberg, H., Myers, M.G., 2009. Leptin acts via leptin receptor-expressing lateral hypothalamic neurons to modulate the mesolimbic dopamine system and suppress feeding. *Cell Metabolism* 10, 89–98.
- Lu, J., Bjorkum, A.A., Xu, M., Gaus, S.E., Shiromani, P.J., Saper, C.B., 2002. Selective activation of the extended ventrolateral preoptic nucleus during rapid eye movement sleep. *J. Neurosci.* 22, 4568–4576.
- Luppi, P.-H., Gervasoni, D., Verret, L., Goutagny, R., Peyron, C., Salvert, D., Leger, L., Fort, P., 2006. Paradoxical (REM) sleep genesis: the switch from an aminergic-cholinergic to a GABAergic-glutamatergic hypothesis. *J. Physiol. Paris* 100, 271–283.
- Luppi, P.-H., Peyron, C., Fort, P., 2016. Not a single but multiple populations of

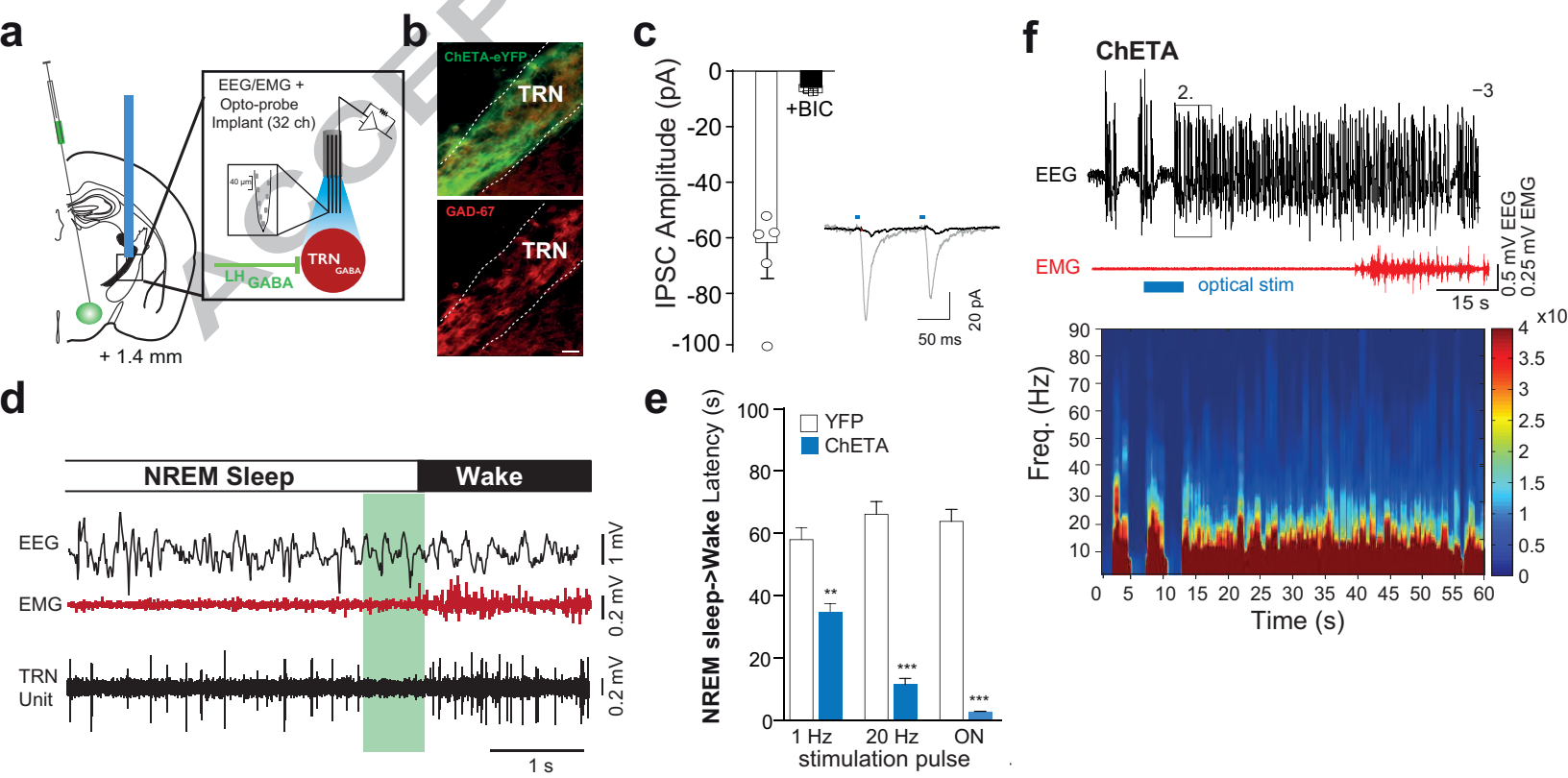
- GABAergic neurons control sleep. *Sleep Medicine Reviews. In Press.*
- Lutter, M., Krishnan, V., Russo, S.J., Jung, S., McClung, C.A., Nestler, E.J., 2008. Orexin signaling mediates the antidepressant-like effect of calorie restriction. *J. Neurosci.* 28, 3071–3075.
- Manns, I.D., Alonso, A., Jones, B.E., 2000. *J. Neurosci.* 20, 1505–1518.
- Nieh, E.H., Matthews, G.A., Allsop, S.A., Presbrey, K.N., Leppla, C.A., Wichmann, R., Neve, R., Wildes, C.P., Tye, K.M., 2015. Decoding Neural Circuits that Control Compulsive Sucrose Seeking. *Cell* 160, 528–541.
- OLDS, J., MILNER, P., 1954. Positive reinforcement produced by electrical stimulation of septal area and other regions of rat brain. *J Comp Physiol Psychol* 47, 419–427.
- Pissios, P., 2009. Animals models of MCH function and what they can tell us about its role in energy balance. *Peptides* 30, 2040–2044.
- Pissios, P., Maratos-Flier, E., 2003. Melanin-concentrating hormone: from fish skin to skinny mammals. *Trends Endocrinol. Metab.* 14, 243–248.
- Presse, F., Conductier, G., Rovere, C., Nahon, J.L., 2014. The melanin-concentrating hormone receptors: neuronal and non-neuronal functions. *Int J Obes Suppl* 4, S31–6.
- Rosin, D.L., Weston, M.C., Sevigny, C.P., Stornetta, R.L., Guyenet, P.G., 2003. Hypothalamic orexin (hypocretin) neurons express vesicular glutamate transporters VGLUT1 or VGLUT2. *J. Comp. Neurol.* 465, 593–603.
- Sakurai, T., 2014. The role of orexin in motivated behaviours. *Nat. Rev. Neurosci.* 15, 719–731.
- Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R.M., Tanaka, H., Williams, S.C., Richardson, J.A., Kozlowski, G.P., Wilson, S., Arch, J.R., Buckingham, R.E., Haynes, A.C., Carr, S.A., Annan, R.S., McNulty, D.E., Liu, W.S., Terrett, J.A., Elshourbagy, N.A., Bergsma, D.J., Yanagisawa, M., 1998. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92, 573–585.
- Sakurai, T., Nagata, R., Yamanaka, A., Kawamura, H., Tsujino, N., Muraki, Y., Kageyama, H., Kunita, S., Takahashi, S., Goto, K., Koyama, Y., Shioda, S., Yanagisawa, M., 2005. Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* 46, 297–308.
- Saper, C.B., Cano, G., Scammell, T.E., 2005. Homeostatic, circadian, and emotional regulation of sleep. *J. Comp. Neurol.* 493, 92–98.
- Saper, C.B., Fuller, P.M., Pedersen, N.P., Lu, J., Scammell, T.E., 2010. Sleep State Switching. *Neuron* 68, 1023–1042.
- Sapin, E., Lapray, D., Bérrod, A., Goutagny, R., Leger, L., Ravassard, P., Clément, O., Hanriot, L., Fort, P., Luppi, P.-H., 2009. Localization of the brainstem GABAergic neurons controlling paradoxical (REM) sleep. *PLoS ONE* 4, e4272.
- Schmidt, M.H., 2014. The energy allocation function of sleep: A unifying theory of sleep, torpor, and continuous wakefulness. *Neurosci Biobehav Rev* 47C, 122–153.
- Schöne, C., Apergis-Schoute, J., Sakurai, T., Adamantidis, A., Burdakov, D., 2014. Coreleased orexin and glutamate evoke nonredundant spike outputs and computations in histamine neurons. *Cell Rep* 7, 697–704.
- Schwartz, M.W., Woods, S.C., Porte, D., Seeley, R.J., Baskin, D.G., 2000. Central nervous system control of food intake. *Nature* 404, 661–671.
- Seeley, R.J., Woods, S.C., 2003. Monitoring of stored and available fuel by the CNS: implications for obesity. *Nat. Rev. Neurosci.* 4, 901–909.

- Segal-Lieberman, G., Bradley, R.L., Kokkotou, E., Carlson, M., Trombly, D.J., Wang, X., Bates, S., Myers, M.G., Flier, J.S., Maratos-Flier, E., 2003. Melanin-concentrating hormone is a critical mediator of the leptin-deficient phenotype. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10085–10090.
- Sherwood, A., Holland, P.C., Adamantidis, A., Johnson, A.W., 2015. Deletion of Melanin Concentrating Hormone Receptor-1 disrupts overeating in the presence of food cues. *Physiol. Behav.* 152, 402–407.
- Sherwood, A., Wosiski-Kuhn, M., Nguyen, T., Holland, P.C., Lakaye, B., Adamantidis, A., Johnson, A.W., 2012. The role of melanin-concentrating hormone in conditioned reward learning. *Eur. J. Neurosci.* 36, 3126–3133.
- Siegel, J.M., 2008. Do all animals sleep? *Trends in Neurosciences* 31, 208–213.
- Stamatakis, A.M., Van Swieten, M., Basiri, M.L., Blair, G.A., Kantak, P., Stuber, G.D., 2016. Lateral Hypothalamic Area Glutamatergic Neurons and Their Projections to the Lateral Habenula Regulate Feeding and Reward. *J. Neurosci.* 36, 302–311.
- Sternson, S.M., 2013. Hypothalamic survival circuits: blueprints for purposive behaviors. *Neuron* 77, 810–824.
- Stratford, T.R., Wirtshafter, D., 2013. Injections of muscimol into the paraventricular thalamic nucleus, but not mediodorsal thalamic nuclei, induce feeding in rats. *Brain Res.* 1490, 128–133.
- Stuber, G.D., Wise, R.A., 2016. Lateral hypothalamic circuits for feeding and reward. *Nat. Neurosci.* 19, 198–205.
- Szymusiak, R., Satinoff, E., 1981. Maximal REM sleep time defines a narrower thermoneutral zone than does minimal metabolic rate. *Physiol. Behav.* 26, 687–690.
- Tritsch, N.X., Ding, J.B., Sabatini, B.L., 2012. Dopaminergic neurons inhibit striatal output through non-canonical release of GABA. *Nature* 490, 262–266.
- Tsunematsu, T., Ueno, T., Tabuchi, S., Inutsuka, A., Tanaka, K.F., Hasuwa, H., Kilduff, T.S., Terao, A., Yamanaka, A., 2014. Optogenetic manipulation of activity and temporally controlled cell-specific ablation reveal a role for MCH neurons in sleep/wake regulation. *J. Neurosci.* 34, 6896–6909.
- Van Cauter, E., Spiegel, K., Tasali, E., Leproult, R., 2008. Metabolic consequences of sleep and sleep loss. *Sleep Med.* 9 Suppl 1, S23–8.
- van den Pol, A.N., Acuna-Goycolea, C., Clark, K.R., 2004. Physiological properties of hypothalamic MCH neurons identified with selective expression of reporter gene after recombinant virus infection. *Neuron* 42, 635–652.
- Vazey, E.M., Aston-Jones, G., 2014. Designer receptor manipulations reveal a role of the locus coeruleus noradrenergic system in isoflurane general anesthesia. *Proc. Nat. Acad. Sci. USA* 111, 3859–3864.
- Verret, L., Goutagny, R., Fort, P., Cagnon, L., Salvert, D., Leger, L., Boissard, R., Salin, P., Peyron, C., Luppi, P.-H., 2003. A role of melanin-concentrating hormone producing neurons in the central regulation of paradoxical sleep. *BMC Neurosci* 4, 19.
- Vyazovskiy, V.V., Olcese, U., Hanlon, E.C., Nir, Y., Cirelli, C., Tononi, G., 2011. Local sleep in awake rats. *Nature* 472, 443–447.
- Waterson, M.J., Horvath, T.L., 2015. Neuronal Regulation of Energy Homeostasis: Beyond the Hypothalamus and Feeding. *Cell Metabolism* 22, 962–970.
- Willie, J.T., Sinton, C.M., Maratos-Flier, E., Yanagisawa, M., 2008. Abnormal response of melanin-concentrating hormone deficient mice to fasting: hyperactivity and rapid eye movement sleep suppression. *Neuroscience* 156, 819–

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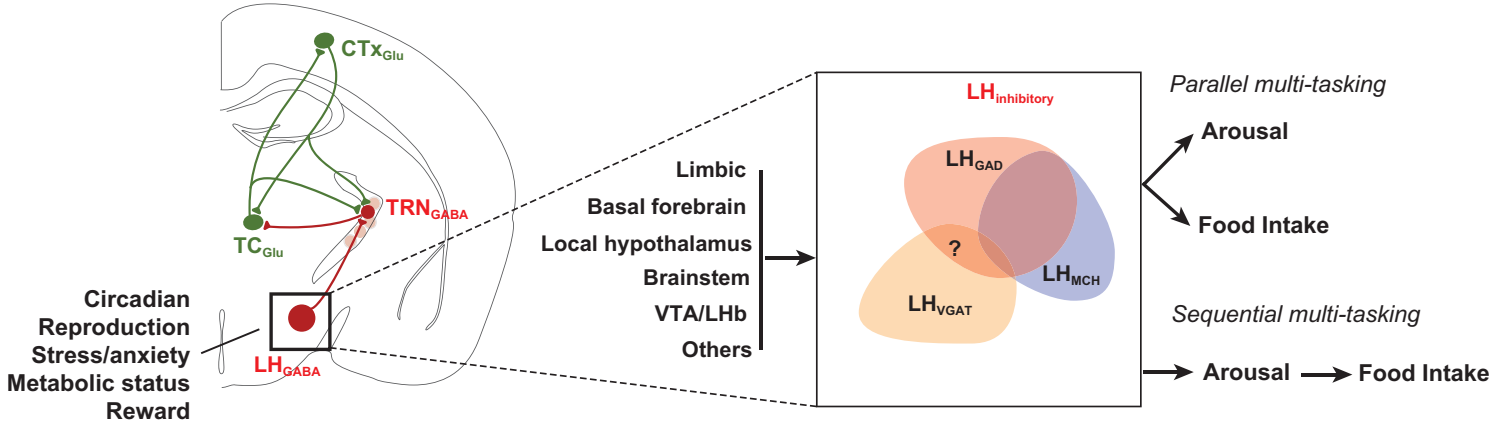
- Yamanaka, A., Beuckmann, C.T., Willie, J.T., Hara, J., Tsujino, N., Mieda, M., Tominaga, M., Yagami, K.-I., Sugiyama, F., Goto, K., Yanagisawa, M., Sakurai, T., 2003. Hypothalamic orexin neurons regulate arousal according to energy balance in mice. *Neuron* 38, 701–713.
- Zhang, Z., Ferretti, V., Güntan, İ., Moro, A., Steinberg, E.A., Ye, Z., Zecharia, A.Y., Yu, X., Vyssotski, A.L., Brickley, S.G., Yustos, R., Pillidge, Z.E., Harding, E.C., Wisden, W., Franks, N.P., 2015. Neuronal ensembles sufficient for recovery sleep and the sedative actions of $\alpha 2$ adrenergic agonists. *Nat. Neurosci.* 18, 553–561.
- Ziegler, D.R., Cullinan, W.E., Herman, J.P., 2002. Distribution of vesicular glutamate transporter mRNA in rat hypothalamus. *J. Comp. Neurol.* 448, 217–229.





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Figure 3 - Herrera et al



Highlights

- Hypothalamus circuits support physiological regulation of homeostatic processes.
- Both peptidergic and GABA/Glutamate neurons from the lateral hypothalamus regulate metabolism and sleep-wake states.
- How single hypothalamic circuit controls sleep and metabolism remains unclear.
- The functional connectivity of sleep and metabolic circuits awaits further investigation.

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